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Different Physiological MRI Noise Between Cortical Layers

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Significantly higher temporal fluctuations of the blood oxygenation level-dependent (BOLD) signal in the living rat group compared to that in the dead rat group were observed in the cortex, suggesting the existence of physiological information in the signal fluctuations. A similar analysis shows significantly different fluctuations between visual cortical layers. The highest fluctuations were observed in layers 4 and 5 and the lowest in layer 1. Given the consistency with published electrophysiology studies anticipating high spontaneous activity in the deeper layers (particularly layer 4), and low activity in superficial layers, we hypothesize that the BOLD signal temporal fluctuations may reflect cortical neuronal activity. Temporal fluctuations in ultrahigh spatial resolution data of the rat brain were measured in two ways. In the first, analyses were performed according to known layer widths, and in the second equal lines of 117 μ along the cortex were selected. The second approach yielded temporal fluctuations along the cortex that resemble known neuronal density distributions including the intralayer structure, particularly within layer 5. Magn Reson Med 52:913–916, 2004.

Key words: physiological noise; fMRI; BOLD; cortex

Functional MRI (fMRI) using blood oxygenation level-dependent (BOLD) contrast is believed to be coupled to neuronal activity through changes in flow and metabolism (1). However, the exact relationship between these parameters and the relative change in the BOLD signal is not clear. Achieving higher fMRI temporal and spatial resolution is a widespread objective that might also help increase our understanding of the nature of the BOLD signal. Recently, it was shown that high field MRI is sensitive enough to detect laminar functional activity in the rat somatosensory cortex (2) as well as small neuronal compartments in the different glomerulus of the rat olfactory bulb (3). Here, we further examine BOLD sensitivity during spontaneous activity.

The six different cortical layers in the visual cortex have various cell bodies, synapses, and dendrite densities. They receive their projections from diverse brain areas and have different spontaneous activity characteristics. For example, the deeper layers have higher spontaneous neuronal activity and lower synaptic density than the superficial layers. In order to measure the spontaneous activity of the different cortical layers, the conventional (On-Off) measurement used in fMRI cannot be applied since stimulation causes unequal neuronal activity in the different layers (4,5). In addition, previous studies have shown that during stimulation the BOLD signal is strongly affected by the high blood vessel density in the brain superficial layer (2,6). Therefore, a different approach is required. Extraction of physiological information from the BOLD temporal fluctuations has been used to measure functional connectivity between different cerebral volumes in normal and pathological conditions (7–11). It was found that the low frequencies of these temporal fluctuations, after cardiac and respiratory filtering, could be used to identify regions of common activity, thus indicating areas that are functionally connected. In addition, the level of these fluctuations was shown to change with TE and signal strength, similar to BOLD changes, suggesting a common mechanism for both (12). In this study we adopted the functional connectivity approach that uses BOLD temporal fluctuations to measure the average cortical activity together with ultrahigh spatial resolution images to assess the spontaneous activity of the different cortical layers.

MATERIALS AND METHODS

All experimental procedures complied with Institutional Animal Care and Use Committee guidelines and were carried out with the approval of the Hebrew University Faculty of Medicine and Hadassah Medical Organization.

Seven male Sprague-Dawley rats, 250–300 g, were used in this study. Rats were anesthetized with ketamine (90 mg/kg i.p.) and xylazine (5 mg/kg i.p) and were restrained in a home-built head and body holder. Temperature was maintained with a water blanket. MRI measurements were performed on a 4.7 T Bruker Biospec system, using a 38-mm Bruker head-dedicated volume coil. An echo planner imaging (EPI) sequence was applied to obtain two coronal slices, TR = 2000 ms, TE = 25 ms, matrix size = 256 × 128 zero-filled to 256 × 256, FOV = 3 × 3 cm, 2 slices approximately –7.0 AP (13), 1 mm slice thickness. Each dataset consisted of 100 images with a temporal resolution of 2 sec. For each animal, three to five sets were collected, then a lethal dose of pentobarbital (120 mg/kg i.p.) was given and the protocol was repeated.

Image analysis was performed using self-written software, in IDL (Interactive Data Language, Research System, Boulder, CO). The first 10 images of each dataset were removed to ensure steady state, and a low-pass filter (0.15 Hz) was applied to the remaining images to minimize the influence of cardiac and respiratory fluctuations on the measured fluctuations, given that the cardiac rate was 2.6 ± 0.1 Hz and the respiratory rate was 1.33 ± 0.16 Hz, as measured using Bruker respiratory and ECG sensors. We

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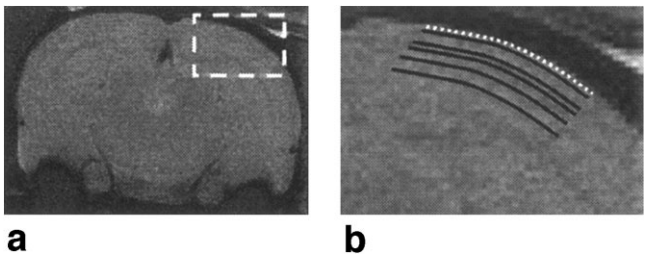

FIG. 1. Illustration of cortical layer selection. **a:** Coronal slice covering the rat visual cortex area. **b:** Expansion of the square marked by the white dashed line in (**a**) covering the rat visual cortex. The white dotted line indicates the brain curvature chosen by the user, whereas the black lines indicate the different layers for the analysis.

are aware that such filtering cannot completely remove these fluctuations and only minimize its effect. The temporal fluctuation from the different ROIs (either a cortical layer or a line (of 117 μ) within the layer) was calculated by averaging all voxel temporal standard deviations. The ROI was semiautomatically chosen, as demonstrated in Fig. 1. A coronal slice covering the rat visual cortex is shown, with the white dashed square marking the area from which data were taken (Fig. 1a). The brain curvature (demonstrated by the white dotted line in Fig. 1b) was marked manually, and the program automatically chose the correct ROIs for 1) the different cortical layers based on layer width taken from Swanson's rat brain atlas in the right and the left cortex (13), and 2) the different lines of 117 μ in width along the cortex.

To allow for comparison across rats, the data were normalized by the whole brain intensity. Finally, a paired *t*-test between the different layers was performed together with a comparison of the dead rat data. Differences in temporal fluctuations between layers and between living and dead animals were observed for each rat individually; however, due to high variance between animals probably due to anesthesia, a paired *t*-test was used. Therefore, the comparison in Figs. 2 and 3 is presented after subtracting the mean temporal fluctuation of each rat from its data. In this way, the standard errors in the figures are comparable to the significance calculated by the paired *t*-test. The first point in these figures (either layer 1 or the first point in Fig. 3) was given the value "1" to allow the reader to estimate the fraction of change in the different layers. Note that this does not mean normalization to the first point and therefore the figure has error bars. This comparatively complex representation was used instead of the more natural, temporal over the mean representation, since the latter biases the results. For example, due to partial volume in layer 1 its mean signal is lower, resulting in high fluctuations over the mean even for a dead rat where equal value is expected. Table 1 summarizes the fluctuations and the mean signal in all layers allowing estimation of the fluctuation level. Note the large standard deviations in this table resulting from differences in anesthesia, as discussed above.

RESULTS

Figure 2 shows the mean temporal SD of the cortical layers relative to layer 1 calculated in the right hemisphere for each animal separately and averaged together with gray bars for living rats and black bars for dead rats. No significant differences between right and left hemispheres were found; thus, results from only one hemisphere is shown. As expected, the temporal fluctuations of the dead rats were significantly lower in all layers, besides layer 1 that is known to have minimal spontaneous activity. These fluctuations represent the measurement noise. In living rats, the physiological fluctuations add to this noise, therefore it is always higher or equal to it. Layer 1, consisting mostly of synapses and very few cell bodies, had significantly lower temporal fluctuations compared to layers 2/3, 4, 5, and 6 ($P < 0.01$), layer 4 had significant higher temporal fluctuations compared to layers 1 and layers 2/3 ($P < 0.05$) and layer 5 had significant higher fluctuation compared to layer 6 and layers 2/3 ($P < 0.05$). This suggests higher spontaneous activity in layers 4 and 5, intermediate activity in layers 2/3, and 6 and lower activity in layer 1.

Figure 3 shows the mean temporal fluctuations and the mean signal intensity for each line in the data (width = 117 μ) arranged according to their distance from the cortical surface, after subtraction for each animal of its mean fluctuation or mean signal, respectively. This presentation shows the inner layer structure, which once again demonstrates higher spontaneous activity in layers 4 and 5, lower at layers 2/3 and 6, and much lower in layer 1. For purposes of comparison, the mean signal is also shown along the cortex. As can be seen, temporal fluctuations along the cortex is connected to the mean signal, as expected, due to spontaneous modulation in the deoxyhemoglobin level that causes modulation in relaxation, and hence signal intensity. The correlation fails when other factors affect the mean, as is the case in layer 1 where partial volume lowers the signal (Table 1). The figure demonstrates the higher sensitivity to neuronal activity of the temporal fluctuation, in that: 1) Layer 6 is distinct from the other layers in terms of the temporal fluctuation measure but not the mean, 2) the inner layer structure is only seen in the temporal fluctuation measure, and 3) layers are clearly

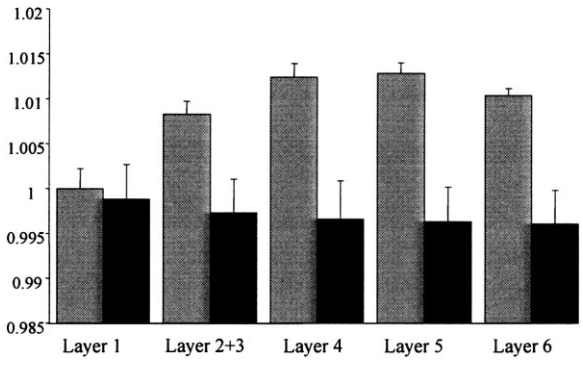

FIG. 2. Average BOLD temporal fluctuations of the different cortical layers. Data were averaged for seven rats with standard error representing the averaged variance calculated for each rat individually (see Materials and Methods). Fluctuations of the living rats (gray bars) are compared to the dead rats (black bars). Note that the signal is normalized to the mean fluctuation and the vertical scale is shifted such that the first point has the value of "1," as described in Materials and Methods.

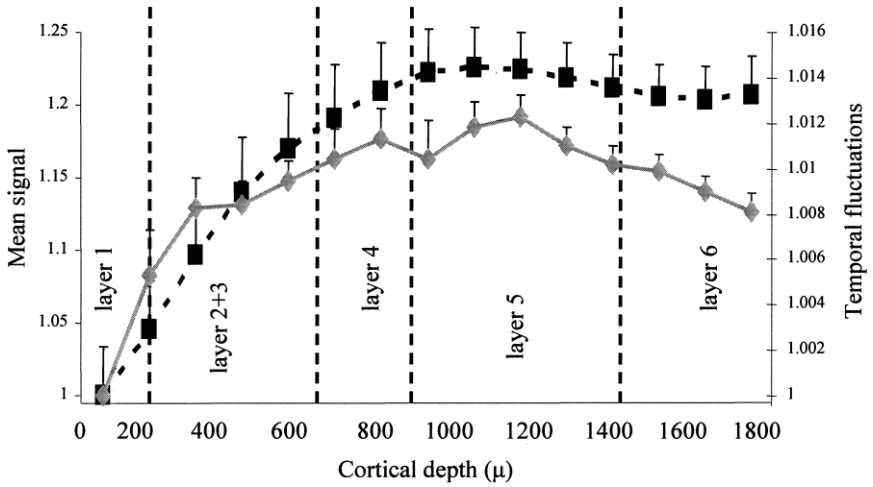

FIG. 3. Average temporal fluctuations and mean signal intensity from 117 μ lines along the right visual cortex with their standard errors. In addition to resemblances to known anatomical layers (dashed lines), interlayer patterns are also observed (gray solid line, right axis). The mean BOLD signal (black dashed lines, left axis) does not show differences between layers besides layer 1 and layer 2/3 that are different from layers 4, 5, and 6 and does not show interlayer structure.

seen in the temporal fluctuation function along the cortex, whereas the mean function is smooth.

Figure 4 shows 2D temporal fluctuation maps of a living and dead brain of a representative rat with a color scale comparable to Fig. 2. To make differentiation between layers easier, a 1D temporal fluctuation function along the cortex was added to the figure, demonstrating maximum fluctuations in deeper layers. The figure highlights the significant difference between the same animal when alive and when dead. It illustrates the complexity of fMRI signal fluctuations and the fact that it can indeed be generated by spontaneous cortical activity and/or by low-frequency vascular modulation. Besides distinct areas of high contrast in the raw images, the dead brain map is uniform, with low levels of fluctuations. The fluctuations in the living brain map have a high average signal in the brain and a lower signal outside the brain. Clearly, the average temporal fluctuation in the living brain (Fig. 4b) is higher than the average fluctuation in the dead brain (Fig. 4c); however, muscle area outside the brain which obviously has no spontaneous neuronal activity shows similar temporal fluctuation, probably reflecting its vascular modulation. Also note that the small differences between the temporal fluctuations (~1–1.5%, Fig. 2) in the group layers are not directly mapped in this individual example.

DISCUSSION

The significant higher temporal fluctuations of the living rat as compared to the dead rat demonstrate that temporal fluctuations contain physiological information. Hence, different cortical volumes should show different fluctuations, reflecting their unique properties. This conclusion is independent of the source of the temporal fluctuations, which we are currently unable to resolve; i.e., if fluctuations are linked to a greater extent to spontaneous cortical activity or to low frequency vascular modulation resulting from cerebral blood flow regulation. However, the findings that layers 4 and 5 generally have the highest temporal fluctuations while layer 1 has the lowest is highly concordant with current knowledge suggesting that the fluctuations should be interpreted as cortical activity and not as blood flow regulations. Cell recordings in the rat somatosensory cortex have demonstrated a high spontaneous firing rate in cortical layers 4 and 5 (14) and high synaptic activity in layers 2/3 and 4 (15), whereas layer 1 is typically expected to have the lowest spontaneous firing rate activity. Furthermore, the functional dependence of the temporal fluctuations along the cortex (Fig. 3), in which differences between and within layers are observed, resembles the neuron density along the cortex, including the neuron density variation within layer 5 (16). Thus, overall, temporal fluctuations appear to be good indicators of cortical activity and their sensitivity is superior to mean signal measurement.

Figures 2, 3 show the lower temporal fluctuations in layer 1 compared to the other layers. It might be claimed that this difference is artificial because it results from partial volume distortions by the superficial rich blood vessel volume that is proximate to the layer, and in particular to the layer width and the experimental spatial resolution. However, the partial volume contribution from noncapillary blood vessels that account for fluctuations resulting from blood flow modulation is expected to in-

Table 1
Mean and temporal fluctuations of alive and dead rats with $\pm\sigma$ of the whole set

	Layer 1	Layer 2 + 3	Layer 4	Layer 5	Layer 6
Alive rats:					
Mean signal	0.664 ± 0.20	0.8423 ± 0.25	0.978 ± 0.26	1.030 ± 0.22	1.030 ± 0.18
Temporal fluctuations	0.1125 ± 0.01	0.1201 ± 0.02	0.1229 ± 0.02	0.1199 ± 0.02	0.1187 ± 0.01
Dead rats:					
Mean signal	0.9462 ± 0.18	1.0958 ± 0.17	1.1264 ± 0.19	1.0874 ± 0.20	1.103 ± 0.18
Temporal fluctuations	0.1068 ± 0.02	0.1052 ± 0.02	0.1040 ± 0.02	0.1052 ± 0.02	0.1044 ± 0.02

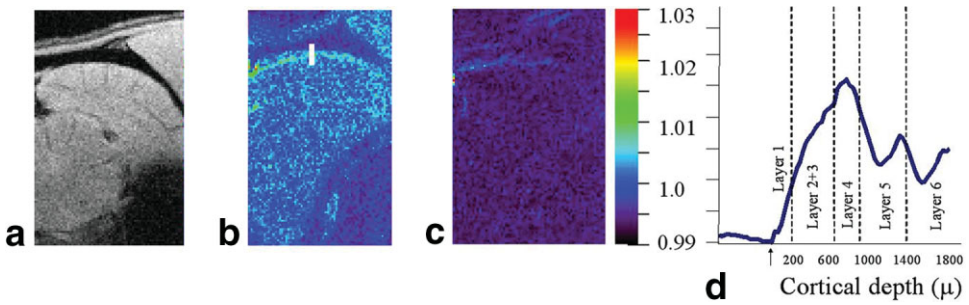

FIG. 4. BOLD temporal fluctuations maps of living and dead brain. **a:** The reference image showing the visual cortex. **b:** Living rat brain and (**c**) dead rat brain. Global temporal fluctuations reduction in the dead brain (**c**) is clearly seen compared with the living one. Due to the time-difference of anatomical slice acquisition it is slightly tilted, probably due to motion. **d:** A 1D projection of the white line in (**b**) showing fluctuation differences between layers. Note that the vertical scale is identical to the color-bar scale.

crease the BOLD signal (2), thus also increases its variance. Our findings show decreased fluctuations, hence indicating immunity to partial volume effect.

The data in Figures 2, 3 were taken from rats anesthetized with ketamine; however, since anesthesia itself affects the basal neuronal activity, we tested whether the relative differences between temporal fluctuations of the different layers are about constant or whether they were modulated by the anesthesia used. Two additional groups of rats were anesthetized, either with urethane (1 g/kg) or with a mixture of isoflurane with 30:70 O_2 and N_2O, with about equal physiological conditions. No significant differences were observed between different types of anesthesia in terms of the relative fluctuations across layers.

In conclusion, we have demonstrated that using ultra spatial resolution images, the BOLD signal fluctuation is sensitive enough to detect differences in signal temporal fluctuations between cortical layers. The results support our hypothesis that these fluctuations are directly related to neuronal activity. However, further work is needed to firmly separate this from physiological noise effects due to vasculature that is not of neuronal basis. If the former is the case, the method could have clinical significance even when only lower spatial resolution is available in pathological brain disorders that anticipate reduced or increased cortical spontaneous activity, such as in Parkinson's disease. Furthermore, monitoring basal level activity helps in understanding fMRI, due to the relative nature of this measurement.

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